Bacillus sp. DU-106 ameliorates anti-diabetic effect by modulating gut microbiota in high-fat-fed and streptozotocin-induced type 2 diabetic mice

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Research Article

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Abstract

Type 2 diabetes (T2D) is a chronic disease that manifests with endocrine and metabolic disorders, seriously threatening public health. This study aims to investigate the effects of *Bacillus* sp. DU-106 on anti-diabetic and gut microbiota in C57BL/6J mice on a high-fat diet (HFD) and streptozotocin (STZ)-induced T2D. *Bacillus* sp. DU-106 was administered to model mice for 8 consecutive weeks. Oral administration of *Bacillus* sp. DU-106 led to decreased food and water intake and alleviated body weight loss. And *Bacillus* sp. DU-106 imparted several health benefits to the mice, including balanced blood glucose, alleviation of insulin resistance in the T2D mice, and an improvement in lipid metabolism. Furthermore, *Bacillus* sp. DU-106 protected against liver and pancreas impairment to some extent. Additionally, *Bacillus* sp. DU-106 treatment reshaped the intestinal flora by enhancing gut microbial diversity and enriching the abundance of certain functional bacteria. Collectively, these findings suggested that *Bacillus* sp. DU-106 has the potential to ameliorate T2D by regulation of the gut microbiota. Therefore, a novel probiotic proposed here, *Bacillus* sp. DU-106 might be a promising therapeutic agent to prevent and alleviate T2D in mice.

Introduction

Diabetes is a chronic metabolic disease that is caused by environmental and genetic factors and is related to insufficient insulin secretion and dysfunction of glucose metabolism. The International Diabetes Federation reported that more than 400 million people were diagnosed with diabetes in 2017; in addition, they estimated that the global prevalence of diabetes is expected to increase up to 640 million by 2040 [1, 2]. Type 2 diabetes (T2D) is the most common type of diabetes, accounting for more than 90% of diabetic cases worldwide [3]. While genetic factors affect susceptibility, it is also evidenced that T2D is caused by a complex combination of external factors such as obesity, unreasonable diet structure, and a sedentary lifestyle [4]. Commercially available drugs, including biguanides, sulfonylureas, non-sulfonylureas, α-glucosidase inhibitors, and insulin, are used as T2D therapeutic drugs [5]. However, part of these drugs has side effects including hepatotoxicity, hypoglycemia, bloating, and diarrhea [6]. Consequently, it is particularly important to explore new measures for preventing and treating T2D with low side effects.

Increasing evidence suggests that the gut microbiota plays a key role in the development and progression of T2D [7]. Gut microbiota is regarded as a microbial organ that symbiotically operates within the host and helps sustain the integrity of the intestinal wall, develop the immune system, assist in metabolism, and prevent the overgrowth of pathogenic organisms [6]. Maladjustment of the gut microbiota can cause the microbiome or its endotoxins to enter the circulatory system and result in low-grade inflammation [8]. Recent reports have demonstrated intestinal microbiota composition between healthy individuals and T2D patients was significantly different. In the T2D patients, gut microbiota dysbiosis was observed, along with an increase in the Firmicutes/Bacteroidetes ratio and pathogenic bacteria, and a reduction in SCFA-producing bacteria [9, 10]. Gut microbiota dysbiosis can lead to increased intestinal permeability

and altered mucosal immune responses, which may, in turn, cause the development or deterioration of T2D [11]. Therefore, these findings provide the rationales to target the gut microbiota in T2D therapy.

Probiotics are live microorganisms that are viewed as potential alternatives in T2D intervention. When given in adequate amounts, certain probiotics can provide the host with health benefits [12]. Several different strains of probiotics, especially *Lactobacillus* and *Bifidobacterium* [13], have been demonstrated to improve T2D symptoms, but the beneficial effects of probiotics are generally strain-specific and depend on the host's physiology [14]. Notably, the *Bacillus* strains appeared to be more suitable for use as health-promoting formulations than some *Bifidobacterium* and *Lactobacillus* strains [15]. The differences are mainly derived from the aerobic and endospore-forming properties of *Bacillus* species, which allow them to withstand harsh environmental pressures, such as low temperatures and high acidity during storage and processing [16]. However, so far *Bacillus* strains are rarely reported to have hypoglycemic effects.

Bacillus sp. DU-106 is separated from traditional fermented yogurt in our lab. The probiotic features of the tested strain showed the ability to survive in bile salts, acidic pH, and in vitro simulated gastric juice [17]. Huang et al have shown that *Bacillus* sp. DU-106 treatment can alleviate hypercholesterolemia; furthermore, this strain has the potential to impart probiotic benefits to the intestine [18]. Lai et al reported that *Bacillus* sp. DU-106 may activate innate immunity via modulating gut microbiota [19]. Huang et al also found that *Bacillus* sp. DU-106 ameliorates antibiotic-associated diarrhea in mice [20]. However, the effect of *Bacillus* sp. DU-106 on T2D and the regulation of gut microbiota remains poorly reported. In this study, we investigated whether *Bacillus* sp. DU-106 can exert an anti-diabetic effect by adjusting the gut microbiota of T2D mice, where diabetes was induced by a high-fat diet (HFD) and streptozotocin (STZ).

Materials And Methods

Bacterial Strain

Bacillus sp. DU-106, which belongs to the *Bacillus* cereus group, was isolated from the traditionally fermented yogurt and was identified by the 16S rRNA sequencing. The isolated strain was preserved in the Research Center for New Resource Food and Functional Evaluation, South China Agricultural University.

Animal Experiments

Forty male-specific pathogen-free (SPF) mice of grade C57BL/6J weighing 18–22 g were purchased from the Southern Medical University Laboratory Animal Center (Guangdong, China, Permit No: SCXK(Yue)-2018-0002). The mice were housed in a conventional 12-hour light/12-hour dark cycle at a temperature of 24 ± 1°C and humidity of 50 ± 5% in the animal facility at the Guangdong Pharmaceutical University. After a week of acclimatization, the mice were randomly separated into two groups as follows: a normal group (NC) and an HFD/STZ-induced T2D experimental group. The NC group was fed a normal diet (LF10C, containing 10% kcal fat, 20% kcal protein, and 70% kcal carbohydrate), while the

experimental group was fed a high-fat diet (HF60, containing 60% kcal fat, 20% kcal protein, and 20% kcal carbohydrate). On the last day of week 4, all mice fasted for 12 h. The NC group received a citrate buffer, and the experimental group was intraperitoneally injected with 1% STZ (Sigma, St. Louis, MO, USA) dissolved in 50 mmol l^{-1} citrate buffer (pH = 4.2 ~ 4.5); STZ was administered to subjects with doses of 50 mg kg^{-1} of the body weight (bw). On the 7th day upon STZ injection, the mice fasting blood glucose (FBG) level was higher than 11.1 mmol l^{-1} and thus confirmed hyperglycemia.

Upon establishment of the diabetic mice model, the mice were randomly separated into three groups as follows (n = 10 in each group): the diabetic model group (MOD); the *Bacillus* sp. DU-106 group (DC) in which the diabetic mice were orally administered with *Bacillus* sp. DU-106 (10^8 CFU kg^{-1} bw/day); and the metformin group (MC) in which the diabetic mice were orally administered with metformin (200 mg kg^{-1} bw/day). In contrast, the NC group and MOD group were orally administered with normal saline.

Body Weight, Food and Water Intake Measurements

The body weight, food, and water intake of all experimental mice were recorded weekly from weeks 5 to 13. Food and water intake were measured as the total intake per cage, which were defined as the following formula: (total weight of intake per cage)/(mice per cage).

Blood Glucose Levels

FBG was recorded on the last day of weeks 6 and 12. On these days, after 12 hours of fasting, blood samples were collected from the mice's tails with a blood collection needle. The FBG levels were measured with a glycemic meter (Roche Diagnostics, Mannheim, Germany).

Oral Glucose Tolerance Test (OGTT) and Insulin Tolerance Test (ITT)

For the OGTT, the mice were fasted overnight for 12 h and administered 1.5 g kg^{-1} glucose in week 12. Their blood glucose levels were measured at 0, 15, 30, 60, and 120 min after glucose administration. For the ITT, the mice were fasted for 6 h and then intraperitoneally injected with 0.5 U kg^{-1} of body weight insulin the day before the test completion. The blood glucose levels were determined at 0, 15, 30, 60, and 120 min after the insulin injection. The area under the glucose curve (AUC-glucose) was determined individually from time 0 to 120 min after administration of glucose and insulin for each mouse, and the calculation follows the trapezoidal rule.

Blood and Tissue Sample Collection

At the conclusion of the experiment, all mice spent the night on an empty stomach, followed by being anesthetized (250 mg kg^{-1} 10% chloral hydrate) and sacrificed. Blood samples and colonic contents were collected aseptically from each mouse and kept at − 80°C until assay. The pancreas and liver were quickly removed, washed, weighted, and immobilized in 10% formalin solution. The organ index was calculated as (organ weight/body weight) ×100.

Determination of Biochemical Parameters in Blood

Serum fractions were separated from blood samples upon centrifugation at 3000 g for 15 min under 4℃. The serum lipid levels, including that of triacylglycerols (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured by the commercial kits (Nanjing Jiancheng Biology Engineering Institute) and their corresponding manufacturer's instructions. The serum insulin and glycated hemoglobin (HbA1c) levels were analyzed by an ELISA kit (Cusabio, Wuhan, China). The homeostasis model of insulin resistance (HOMA-IR) was computed as follows: HOMA-IR = fasting insulin level×fasting blood glucose/22.5. The contents of malondialdehyde (MDA) and the activities of superoxide dismutase (SOD) in plasma analyzed using a commercial kit (Nanjing Jiancheng Biology Engineering Institute).

Histopathological Analysis

The liver and pancreas were fixed and embedded in paraffin, cut to a 5 μm thickness, and subjected to hematoxylin-eosin (H&E) staining. Photomicrographs of the liver and pancreas were captured under a 400× magnification.

Gut Microbiota Analysis

The day before the test completion, the mice feces were collected in a sterile tube, frozen in liquid nitrogen, and stored at − 80°C. Whole genome DNA was extracted and DNA purity was determined by electrophoresis on 1% agarose gels. PCR amplification of the V4 region of the bacterial 16SrRNA gene was performed using forward and reverse primers. For detecting, equal amount of 1×loading buffer was combined with PCR products and subjected to electrophoresis on 2% agarose gel. The PCR products between 400 and 600 bp were harvested and purified with GeneJETTM Gel Extraction Kit (Thermo Fisher Scientific Inc, USA) according to the instructions.

Statistical Analyses

Statistical differences between the control and treatment groups were computed by Tukey's test or one-way analysis of variance (ANOVA) with the SPSS 22 software. Data are presented as means ± SD, and significant difference is determined as $p < 0.05$.

Results

Effects of Bacillus sp. DU-106 on Body Weight, Food, and Water Intake

At the beginning of the intervention, the bodyweight of the MOD group was higher than the NC group with a significantly different. The bodyweight of the MOD group then gradually decreased and became similar to that of the NC group at the end of the experiment. After *Bacillus* sp. DU-106 treatment, the bodyweight of mice increased when compared to the MOD group (Fig. 1A). At the same time, the food and water intake of the MOD group was markedly higher than that of the NC group. However, oral administrations of *Bacillus* sp. DU-106 and metformin could reverse these changes, which could decrease the food and

water intake in T2D mice (Fig. 1B-C). These data suggest that *Bacillus* sp. DU-106 treatment could potentially protect against emaciation, polyphagia, and polydipsia in T2D mice.

Effects of Bacillus sp. DU-106 on the Organ Index and Histopathological Assay

The liver and pancreas indexes were markedly increased for the MOD group as compared to the NC group (Fig. 2, $p < 0.05$ or $p < 0.001$), while both *Bacillus* sp. DU-106 and metformin treatments lowered the liver and pancreas index, but there was no significant difference in the pancreas index.

H&E staining indicated that liver histology in the NC group had a well-organized structure, whereas the histological structure of the MOD group was abnormal (Fig. 2A). These lesions mainly include lipid vacuolation, swollen hepatocytes, steatosis, granular degeneration, and large diffuse lipid microvesicles that occupy most of the cytoplasm of hepatocytes; however, oral administrations of *Bacillus* sp. DU-106 and metformin could reverse these damages to some extent. Histological analysis of the pancreas in the NC group showed that the islets exist as round or oval clusters of cells that were scattered among healthy acinar cells with well-defined borders. In comparison, the pancreatic histology of the MOD group had obvious damage, with characteristics including unclear outer tissues and edges of the islets, irregularly arranged pancreatic islet cells, and atrophied islets infiltrated by inflammatory cells (Fig. 2B). However, the T2D mice treated with *Bacillus* sp. DU-106 and metformin both showed significant recovery of the pancreatic tissue structure, such as clear edges of the islets. These findings suggest that *Bacillus* sp. DU-106 can lessen the liver and pancreas damage in T2D mice.

Effects of Bacillus sp. DU-106 on HbA1c、FBG、Insulin Levels, and HOMR-IR

As shown in Fig. 3A, the level of HbA1c in the MOD group was remarkably higher than that of the NC group, while *Bacillus* sp. DU-106 and metformin treatments lowered the HbA1c levels, especially the *Bacillus* sp. DU-106 intervention ($p < 0.001$). The FBG and insulin levels in the MOD group increased significantly compared to those in the NC group, whereas oral administrations of *Bacillus* sp. DU-106 and metformin both effectively reduced the levels of FBG and insulin (Fig. 3B-C, $p < 0.01$ or $p < 0.001$). Notably, the T2D mice treated with *Bacillus* sp. DU-106 had lowered FBG and insulin levels compared to that received metformin. Consistently, the calculated HOMR-IR index was also higher in the MOD group than the NC group, while treatments with *Bacillus* sp. DU-106 and metformin led to a significant reduction in the HOMR-IR index (Fig. 3D, $p < 0.001$).

Effects of Bacillus sp. DU-106 on OGTT and ITT

Upon glucose administration, the blood glucose levels of the MOD group showed a sharp increase at 15 min and its levels at every test time point were significantly higher than those in the NC group, while supplementations of *Bacillus* sp. DU-106 and metformin effectively inhibited the increase in blood glucose levels (Fig. 3E). The total area under the glucose curve (AUC-glucose) over 120 min was taken as the quantitative results of the OGTT. Although the DC and MC groups of the $AUG_{glucose}$ values were higher than that of the NC group, they were strikingly lower than the $AUG_{glucose}$ value of the MOD group (Fig. 3F,

$p < 0.001$). These results suggest that supplementation with *Bacillus* sp. DU-106 can repair the impaired glucose tolerance in T2D mice.

As shown in Fig. 3G, the ability of T2D mice to improve insulin resistance is not as good as that of normal mice; however, both *Bacillus* sp. DU-106 and metformin interventions can improve insulin tolerance of T2D mice. Interestingly, *Bacillus* sp. DU-106 treatment led to better insulin sensitivity at specific time points than metformin. Similarly, the T2D mice had higher $AUC_{insulin}$ as compared to the normal mice (Fig. 3H, $p < 0.001$). As a result of effective treatments with *Bacillus* sp. DU-106 and metformin, the DC and MC groups had markedly lesser the $AUC_{insulin}$ values ($p < 0.05$ or $p < 0.001$). These findings suggested that *Bacillus* sp. DU-106 treatment could exhibit a positive effect on insulin resistance in T2D mice.

Effects of Bacillus sp. DU-106 on Serum Lipids

As shown in Fig. 4A-D, the levels of TC, TG, and LDL-C in serum of the MOD group were markedly higher, while HDL-C levels were lower than that of the NC group ($p < 0.01$ or $p < 0.001$), suggesting that T2D mice may suffer from abnormal lipid metabolism and hyperlipidemia. *Bacillus* sp. DU-106 and metformin treatments adjusted dyslipidemia by reducing TC, TG, and LDL-C levels and by increasing HDL-C levels ($p < 0.01$, $p < 0.05$, or $p < 0.001$); however, as compared to metformin, treatment with *Bacillus* sp. DU-106 resulted in a more desirable change in these parameters

Effects of Bacillus sp. DU-106 on the activities of SOD and MDA

To study the antioxidant effect of *Bacillus* sp. DU-106 on the serum lipids in T2D mice, we investigated the antioxidant capacity by analyzing the activities of relevant enzymes, MDA and SOD (Fig. 4E-F). In comparison with the NC group, MDA levels in the MOD group increased, while SOD activities were markedly reduced ($p < 0.01$ or $p < 0.001$); however, administrations of *Bacillus* sp. DU-106 and metformin showed strong antioxidant activity, as evidenced by declined MDA levels and elevated SOD activities ($p < 0.05$ or $p < 0.01$). Again, the mice that were administered *Bacillus* sp. DU-106 showed better antioxidant activity than those with metformin. These findings were supportive that *Bacillus* sp. DU-106 ameliorated oxidative stress in T2D mice.

Effects of Bacillus sp. DU-106 on Gut Microbiota in T2D Mice

The composition and abundance of gut microbiota in mice feces were analyzed by the bacterial 16S rRNA genes pyrosequencing to evaluate the impact of *Bacillus* sp. DU-106 on T2D mice. A total of 232,135 clean reads were obtained from 28 samples after quality filtration, and an average of 6,273 ± 852 reads were generated per sample. Based on the operational taxonomic unit (OTU), we found that NC, MOD, DC, and MC had 28 common OTUs, whereas unique OTUs were detected by each group (38 for the NC group, 26 for the MOD group, 380 for the DC group, and 49 for the MC group, respectively); these unique OTUs indicated that treatment with *Bacillus* sp. DU-106 increased the functional and microbial diversity in T2D mice (Fig. 5A). We profiled the gut bacterial communities of the groups by measuring

Chao1 and Ace indexes as an indicator of richness and Shannon and Simpson indexes as markers of diversity. Chao1, Ace, and Shannon indexes decreased, while the Simpson index increased in the MOD group as compared to the baseline level ($p < 0.001$). *Bacillus* sp. DU-106 administration restored the microbiota community diversity and richness, suggesting it can enhance the α-diversity of fecal microbiota in T2D mice (Fig. 5B-E, $p < 0.01$, $p < 0.05$ or $p < 0.001$). The principal coordinates analysis (PCoA) was used to analyze β-diversity, which showed the structural differences of the intestinal microbiota among four test groups (Fig. 5F). The NC group was separately clustered from the MOD group. And, the MOD and DC groups also showed a unique clustering of microbial community structure. These results suggest that treatment with *Bacillus* sp. DU-106 can reshape the gut microbial structure in T2D mice.

Effects of Bacillus sp. DU-106 on the Taxonomic Composition of Gut Microbiota

To assess the changes in the intestinal microbiota of the *Bacillus* sp. DU-106 and metformin treatments, we compared their relative abundance by sequencing the four groups at phylum and genus levels (Fig. 6A-C). At the phylum level, the fecal microbiota of all mice mainly consisted of Firmicutes, Bacteroidetes, Proteobacteria, Tenericutes, Actinobacteria, Cyanobacteria, and Verrucomicrobia. The MOD group showed higher abundance in Firmicutes and lower abundance in Bacteroidetes compared with the NC group, thereby increasing the ratio of Firmicutes to Bacteroidetes. Contrarily, *Bacillus* sp. DU-106 intervention reversed this trend to some extent ($p < 0.001$). At the genus level, the abundances of *Ileibacterium* and *Faecalibaculum* were decreased, while that of *Romboutsia*, *Lactobacillus*, and *Staphylococcus* were increased in the MOD group compared with the NC group. However, the genera of *Blautia*, *Ileibacterium*, *Faecalibaculum, Faecalibacterium*, and *unidentified_Lachnospiraceae* were markedly more abundant in the DC group than those in the MOD group, whereas metformin elevated the abundant of *Akkermansia* and *Allobaculum*. In a word, these data show that *Bacillus* sp. DU-106 and metformin can reshape the intestinal microbiome and improve gut dysbiosis in T2D mice.

Correlation Between Gut Microbiota And Biochemical Parameters

To investigate the associations of the gut microbiota with T2D, we run a Spearman's correlation test between the 35 genera and biochemical parameters. As shown in Fig. 6D, *Romboutsia, Staphylococcus*, and *Lactobacillus* were significantly and positively correlated with HbA1c, FBG, insulin, AUC-insulin, MDA, TG, TC, and LDL-C levels, as well as the HOMR-IR index, whereas *Ileibacterium* and *Faecalibaculum* exhibited an inverse correlation. Additionally, *Blautia* and *unidentified_Lachnospiraceae* were strongly negatively associated with HDL-C. Taken together, increased the abundance of *Ileibacterium*, *Faecalibaculum*, *Blautia*, and *unidentified_Lachnospiraceae* could be beneficial for the alleviation of T2D, but *Romboutsia*, *Staphylococcus*, and *Lactobacillus* might be bad for the T2D treatment.

Discussion

T2D is a serious chronic metabolic disease characterized by insulin resistance, metabolic disorders related to glucose and lipid metabolism, as well as gut microbiota dysbiosis [21, 22]. There is evidence of

the beneficial effects of probiotic supplementation on improving T2D and its consequences on health [23]. In this study, we designed a T2D mice model by administering a combination of HFD and STZ. A combination of biochemical indexes and gut microbiota was then used to determine whether *Bacillus* sp. DU-106 was able to offer a degree of protection against the negative effects of diabetes. We found that *Bacillus* sp. DU-106 administration could substantially alleviate the symptoms of hyperglycemia and hyperlipidemia, as well as organ injury in HFD/STZ-induced T2D mice, and the anti-diabetic effects can be explained by the gut microbiota reorganization.

It is well known that polyuria, polyphagia, and polydipsia remain diagnostic hallmarks of T2D sufferers [24]. In our study, the T2D mice did develop serious symptoms of diabetes, such as increased water and food intake and loss of weight. However, the T2D mice that received *Bacillus* sp. DU-106 and metformin showed significant anti-diabetic action by effectively ameliorating the symptoms. Similarly, Wang et al showed that oral administration of *Lactobacillus rhamnosus*, *Bifidobacterium adolescentis*, or *Bifidobacterium bifidum* could reduce the food and water intake of T2D mice [25], which is consistent with our study. These findings corroborate that *Bacillus* sp. DU-106 has the potential to function as an adjuvant in the management of diabetes and alleviate comorbid symptoms in T2D mice.

Hyperglycemia is one of the characteristics of T2D that can lead to diabetic complications such as nephropathy, neuropathy, atherosclerosis, and retinopathy. Ameliorating abnormal glucose metabolism is the fundamental objective of diabetes remission [26]. Several studies have reported that probiotics intervention led to reduced FBG levels [27, 28]. HbA1c is a form of hemoglobin, which is an important indicator of long-term glucose homeostasis [29]. The T2D mice that administration of *Bacillus* sp. DU-106 markedly decreased the FBG and HbA1c levels, suggesting that *Bacillus* sp. DU-106 alleviates the long-term hyperglycemic state. These results agree with the previous study which showed that *L. rhamnosus* CCFM0528 treatment markedly reduced the FBG and HbA1c values in T2D mice [30]. In particular, insulin resistance as an important indicator of T2D, can accelerate the development of T2D and increase the risk of metabolic diseases [31]. An isolated measurement of glucose or insulin levels does not accurately reflect insulin resistance, therefore, the HOMA-IR test is widely utilized to assess insulin resistance [32]. It has been reported that *L. casei* CCFM419 treatment could alleviate insulin resistance and improve insulin sensitivity in T2D mice [33]. In our study, *Bacillus* sp. DU-106 supplementation markedly ameliorated the insulin status and HOMA-IR values in the T2D mice, which is consistent with the findings of Li et al [29]. Overall, these results suggest that *Bacillus* sp. DU-106 partially alleviates insulin resistance. Besides, the previous finding has confirmed a positive correlation between oral glucose tolerance and insulin sensitivity [34]. We also found that oral administration of *Bacillus* sp. DU-106 markedly enhanced glucose tolerance in T2D mice, suggesting that *Bacillus* sp. DU-106 could increase insulin sensitivity in T2D mice, thus regulating the blood glucose level. Consistent with our findings, Li et al found that *L. plantarum* X1 treatment can effectively reverse the symptoms of insulin resistance and glucose tolerance in T2D mice. This may strengthen the conclusion that *Bacillus* sp. DU-106 has an efficient hypoglycemic effect in T2D mice

Dyslipidemia is often linked to hyperglycemic damage and is the main cause of cardiovascular diseases in diabetic patients [35]. Previous studies have shown that probiotics perform their main functions, at least in part, via lipid regulation [36]. *Bacillus* sp. DU-106 administration led to an adjustment in TC, TG, HDL-C, and LDL-C levels, partially regulating dyslipidemia in T2D mice. These results agreed with a previous study, which reported that *L. plantarum* NCU116 improves lipid profiles in T2D mice [37]. Altogether, these results suggest that treatment with *Bacillus* sp. DU-106 can improve dyslipidemia caused by T2D.

Glucotoxicity featuring chronic hyperglycemia is a common characteristic of T2D, which impairs insulin production and secretion by β-cells [38]. The mechanism of glucotoxicity is majorly mediated by oxidative stress, which is recognized as a primary risk driver for the development of T2D [39]. Free radicals are usually produced by glucose oxidation and lipid peroxidation, while antioxidant enzymes, such as SOD, scavenge these free radicals as part of the antioxidant defense system [40]. Therefore, excessive MDA production is indicative of oxidative stress damage. Previous studies showed that consuming 300 g/d of probiotic yogurt can improve the antioxidant status in T2D patients, consistent with our study in the animal models [41]. Furthermore, oxidative stress can damage the cell membrane, which may contribute to impairments of the liver and pancreas in histopathological studies [42, 43]. Damage to the liver and pancreas islets would aggravate the development of T2D. In our study, treatments with *Bacillus* sp. DU-106 and metformin protected and improved impaired liver and pancreas to some extent in T2D mice. A previous study indicated that liver and pancreatic injury could be relieved by oral LC5 (*L. rhamnosus* Y37, *L. casei* CCFM419, *L. plantarum* X1, *L. brevis* CCFM648, *and L. plantarum* CCFM36) [44]. Another study exhibited that *L. casei* CCFM0412 could ameliorate glucose intolerance by preserving the islet cells [45]. These observations indicate that the probiotic strain *Bacillus* sp. DU-106 has a certain protective effect on oxidative damage, thereby improving insulin resistance and reducing the liver and pancreatic injury in T2D mice.

Recent studies have shown that a moderate degree of intestinal flora imbalance could drive the development of T2D [46]. Accumulating evidence indicated that the low bacterial diversity in the gut microbiota was linked to low-grade inflammation and insulin resistance [47]. In our study, T2D suffered from lowered richness and diversity in gut microbiota, which was reversed by the *Bacillus* sp. DU-106 intervention; this observation is corroborated by a previous study [48]. Previous studies have similarly shown that the gut microbiota in T2D mice was markedly different from that in normal mice, and T2D mice have a higher Firmicutes/Bacteroidetes ratio [49]. In the current study, oral administration of *Bacillus* sp. DU-106 could restore the Firmicutes/Bacteroidetes ratio by bringing it closer to the baseline level. And Larsen et al observed that the ratio of Firmicutes to Bacteroidetes was markedly connected with blood glucose levels [50], suggesting that *Bacillus* sp. DU-106 exhibited a hypoglycemic effect. These outcomes together show that *Bacillus* sp. DU-106 can regulate dysbiosis of intestinal microbiota in T2D mice.

Furthermore, at the genus level, the distribution of intestinal bacterial species was remarkably different among the test groups. In the MOD group, the beneficial bacteria such as *Faecalibaculum* and *Ileibacterium* were decreased, while the *Romboutsia*, *Lactobacillus*, and *Staphylococcus* were increased.

Romboutsia, which belongs to the Firmicutes phylum, has been reported to function in fat metabolism [51, 52]. Although *Lactobacillus* is regarded as a probiotic strain, some studies have proved that higher *Lactobacillus* abundance may lead to increased glucose levels [53]. Dong et al observed that the abundance of *Lactobacillus* was substantially higher in the T2D mice as compared to the normal mice, which is in line with our study [54]. Moreover, *Staphylococcus* is a Gram-positive bacteria, which is related to the onset and development of diabetes [55]. Interestingly, in T2D mice population alterations in these bacterias were all reversed by *Bacillus* sp. DU-106 intervention. Spearman's correlation analysis indicated that *Romboutsia*, *Lactobacillus*, and *Staphylococcus* were strikingly positively correlated with hyperglycemia phenotypes, in agreement with previous observation [56]. In addition, *Bacillus* sp. DU-106 treatment also could increase the abundance of *Faecalibaculum*, *Faecalibacterium*, *Ileibacterium*, *Blautia*, and *unidentified_Lachnospiraceae*, consistent with previous reports [57–59]. *Faecalibaculum* and *Faecalibacterium* were identified as the most abundant butyrate-producing bacteria in the human colon, contributing substantially to gut health and improving metabolic dysfunction [60–62]. Previous studies showed that a reduced abundance of *Faecalibaculum* and *Faecalibacterium* is correlated with various disorders, including inflammatory bowel disease and obesity [63, 64]. *Ileibacterium* has been reported to potentially prevent obesity [12]. In the current study, we found that *Faecalibaculum* and *Ileibacterium* showed strongly and negatively correlated with high glucose, insulin resistance, oxidative stress, and lipid metabolism disorders. *Unidentified_Lachnospiraceae* belongs to the family *Lachnospiraceae* and *Lachnospiraceae* was reported to correlate with improvements in glucose tolerance and dyslipidemia [65]. *Blautia* is another bacterial genus that has been linked to diabetes, whereby it can ameliorate glucose and lipid abnormalities to alleviate T2D [66]. We determined by Spearman's correlation test also showed that *unidentified_Lachnospiraceae* and *Blautia* were a strong positive correlation with serum lipid parameters. Taken together, *Bacillus* sp. DU-106 exhibited anti-diabetic effects, which were partly mediated by regulating gut microbiota composition, such as enrichment of benign bacteria and reduction of harmful bacteria.

Conclusions

In conclusion, our investigation confirmed that the probiotic strain *Bacillus* sp. DU-106 exhibits an excellent anti-diabetic effect in T2D mice. The mechanism by which *Bacillus* sp. DU-106 imparts its anti-diabetic effect is partly attributed to the regulation of blood glucose balance and alleviation of insulin resistance, dyslipidemia, and oxidative stress in T2D mice. In addition, the administration of *Bacillus* sp. DU-106 modulated gut microbiota dysbiosis, such as by enriching beneficial bacterial groups and reducing the harmful species. Based on these results, *Bacillus* sp. DU-106 is proposed to be a promising functional food supplement for the prevention and alleviation of T2D. Overall, our findings provide a reasonable basis for future clinical trials assessing the efficacy and usefulness of *Bacillus* sp. DU-106 is a probiotic against T2D.

Declarations

Availability of Data and Materials

Data supporting the results of this study can be from the corresponding author upon reasonable request. The original file of the bacterial V4 16S rRNA data has been deposited in the NCBI Sequence Read Archive as Bioproject number, PRJNA733578.

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Author Contribution

Study concept and design: JY, JL, BD, and PL. Conducted the experiments: JY, JL, QX, and SX. Analysis and interpretation of data: JY, JL, QX, BD, and PL. Writing—original draft preparation: JY, JL, QX, SX, and JJ. Writing—review and editing: JY, JL, JJ, BD, and PL. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Ethics Approval

All animal experiments were carried out as per the Chinese legislation on the use and care of laboratory animals and were carried out at Guangdong Pharmaceutical University (Approval No: SCXK(Yue)-2018-0002). All animal experiments comply with the ARRIVE guidelines.

Conflict of Interest

The authors declare that there are no competing economic interests.

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Figures

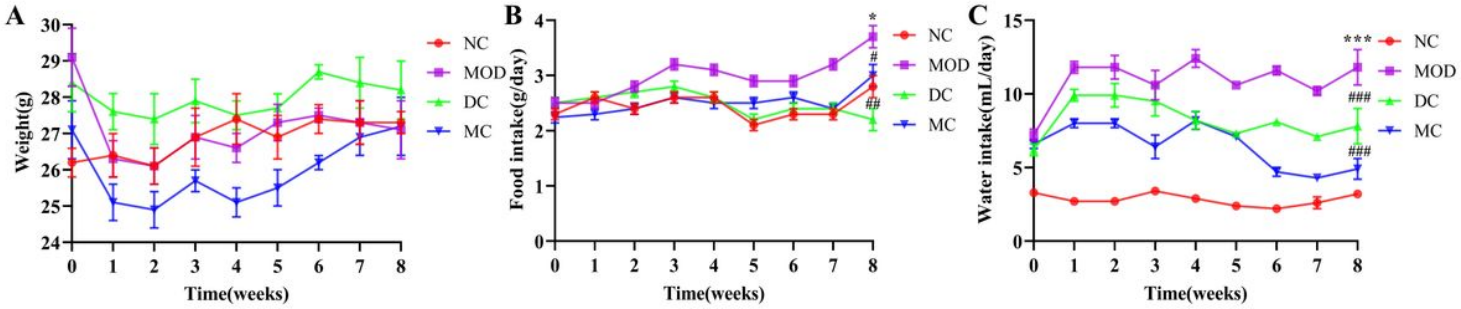

Figure 1

Effects of *Bacillus* sp. DU-106 on the body weight, food, and water intake in T2D mice. (A) Weekly body weight, (B) food intake, and (C) water intake. Data are expressed as mean ± SD (n = 10); * p < 0.05, ** p < 0.01, *** p < 0.001, MOD group vs. NC group; # p < 0.05, ## p < 0.01, ### p < 0.001, DC group or MC group vs. MOD group.

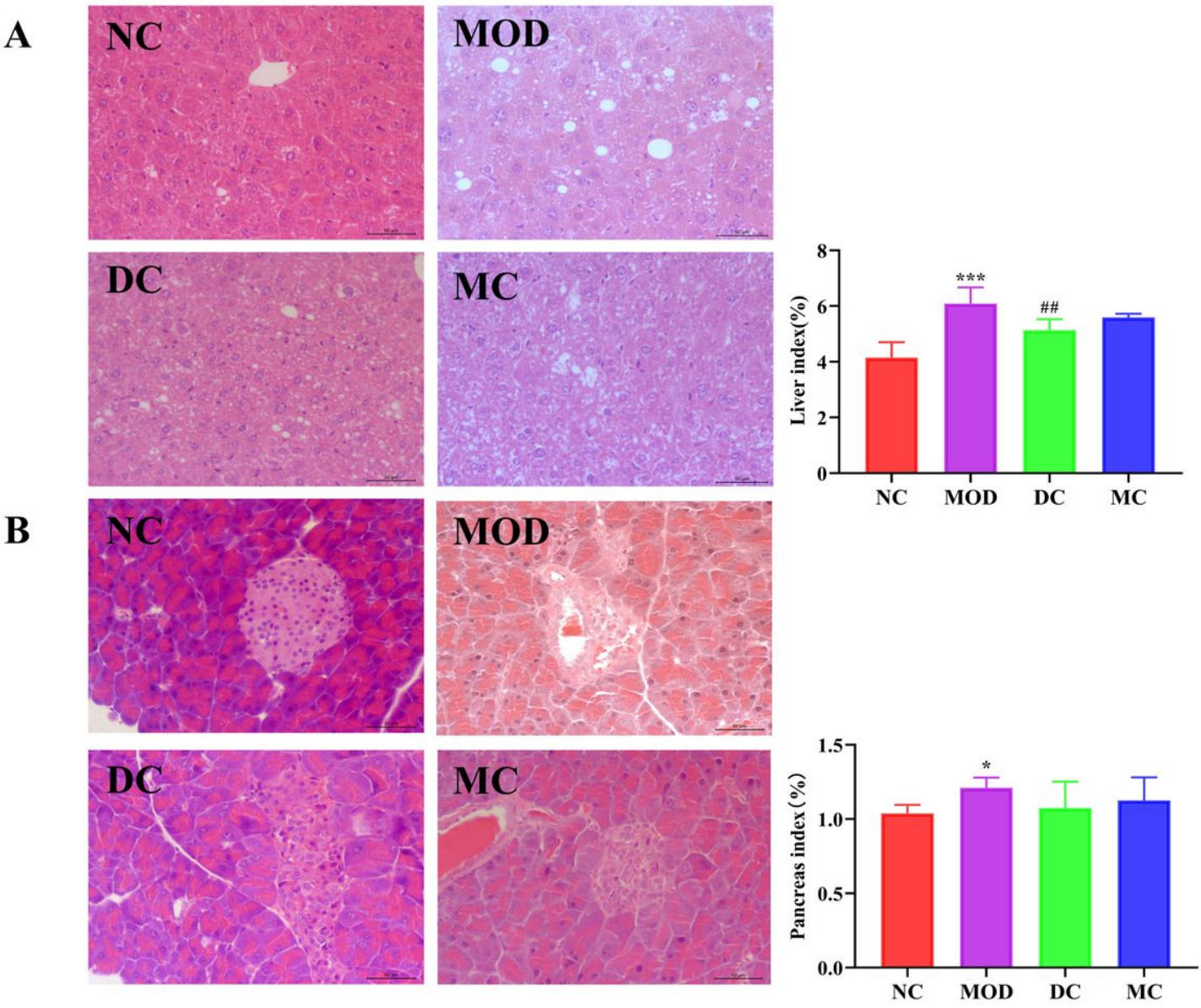

Figure 2

Effects of *Bacillus* sp. DU-106 on the histopathology of liver and pancreas in T2D mice. (A) Liver index and hematoxylin-eosin (H&E) staining of liver (final magnification, 400×), and (B) pancreas index and histopathology of pancreas (final magnification, 400×). Data are expressed as mean ± SD (n = 10); Data are expressed as mean ± SD (n = 10); * p < 0.05, ** p < 0.01, *** p < 0.001, MOD group vs. NC group; # p < 0.05, ## p < 0.01, ### p < 0.001, DC group or MC group vs. MOD group.

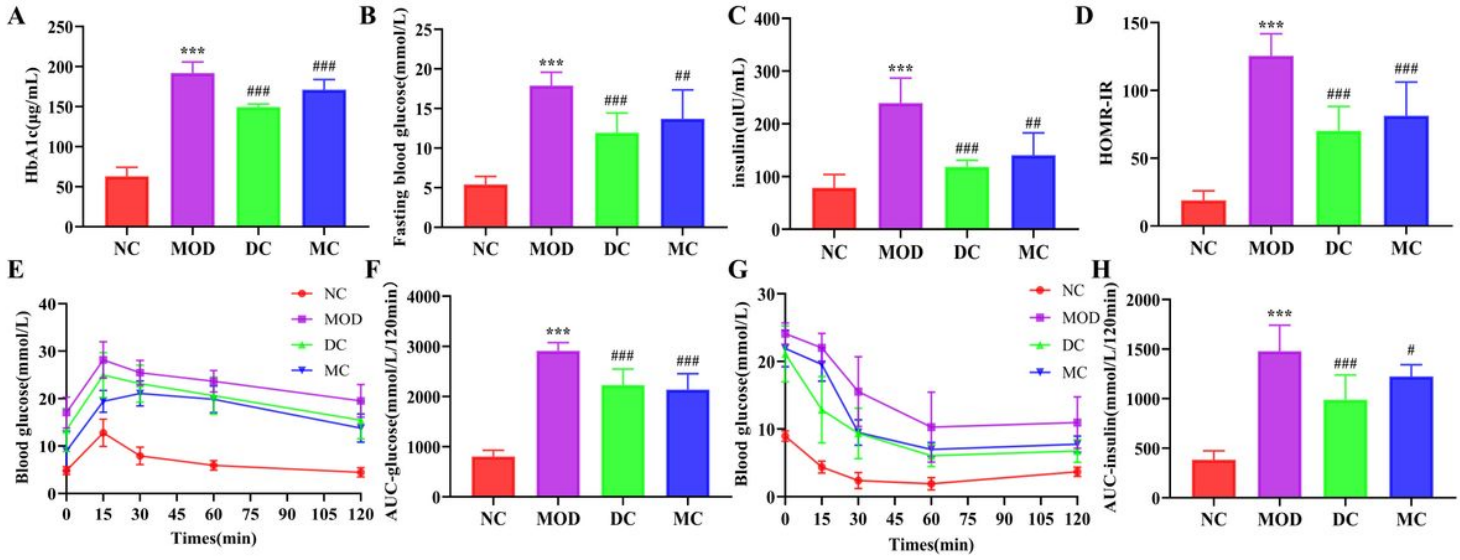

Figure 3

Effects of *Bacillus* sp. DU-106 on serum glucose metabolism disorder in T2D mice. (A) HbAlc level, (B) fasting blood glucose level, (C) insulin level, (D) HOMR-IR index, (E) oral glucose tolerance test, (F) AUC-glucose, (G) insulin tolerance test, and (H) AUC-insulin in mice. Data are expressed as mean ± SD (n = 10); * p 0.05, ** p 0.01, *** p 0.001, MOD group vs. NC group; # p 0.05, ## p 0.01, ### p 0.001, DC group or MC group vs. MOD group.

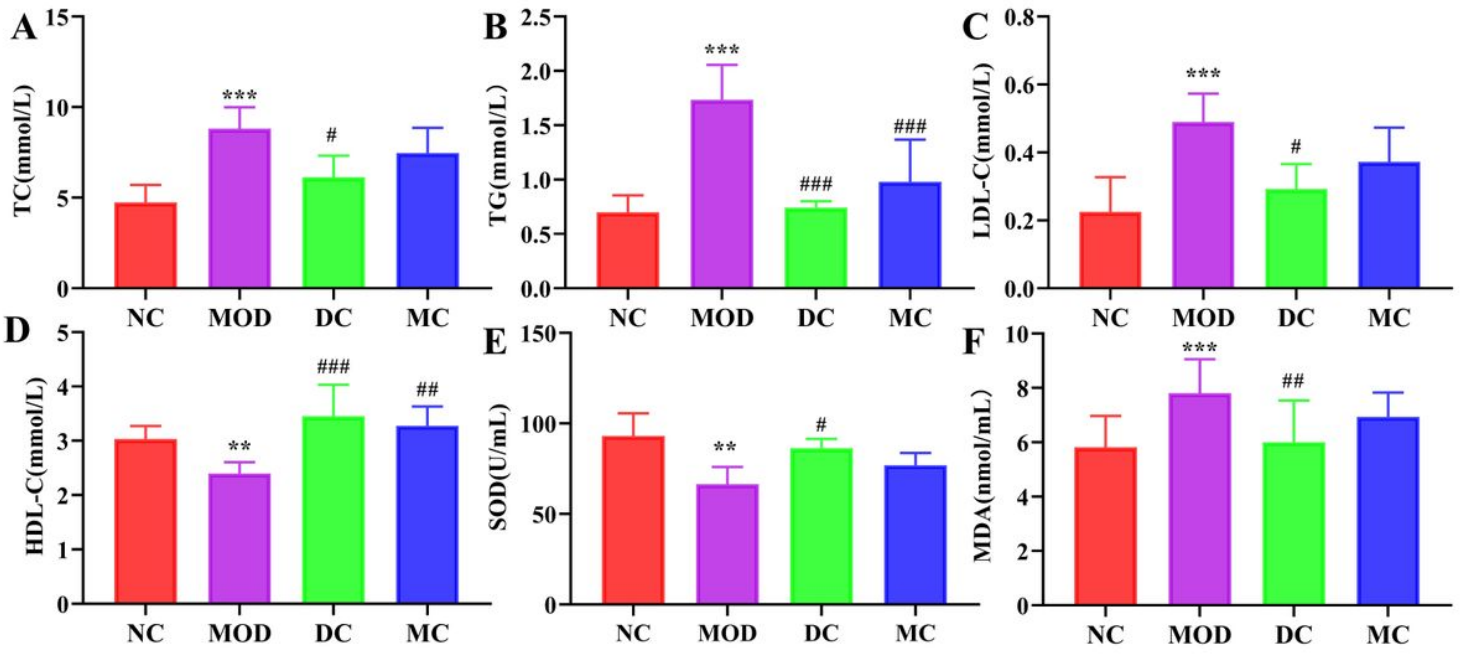

Figure 4

Effects of *Bacillus* sp. DU-106 on serum lipids amd oxidative stress in T2D mice. (A) TC, (B) TG, (C) LDL-C, (D) HDL-C, (E) SOD, and (F) MDA. Data are expressed as mean ± SD (n = 10); * p 0.05, ** p 0.01, ***

p ≤ 0.001, MOD group vs. NC group; # *p* ≤ 0.05, ## *p* ≤ 0.01, ### *p* ≤ 0.001, DC group or MC group vs. MOD group.

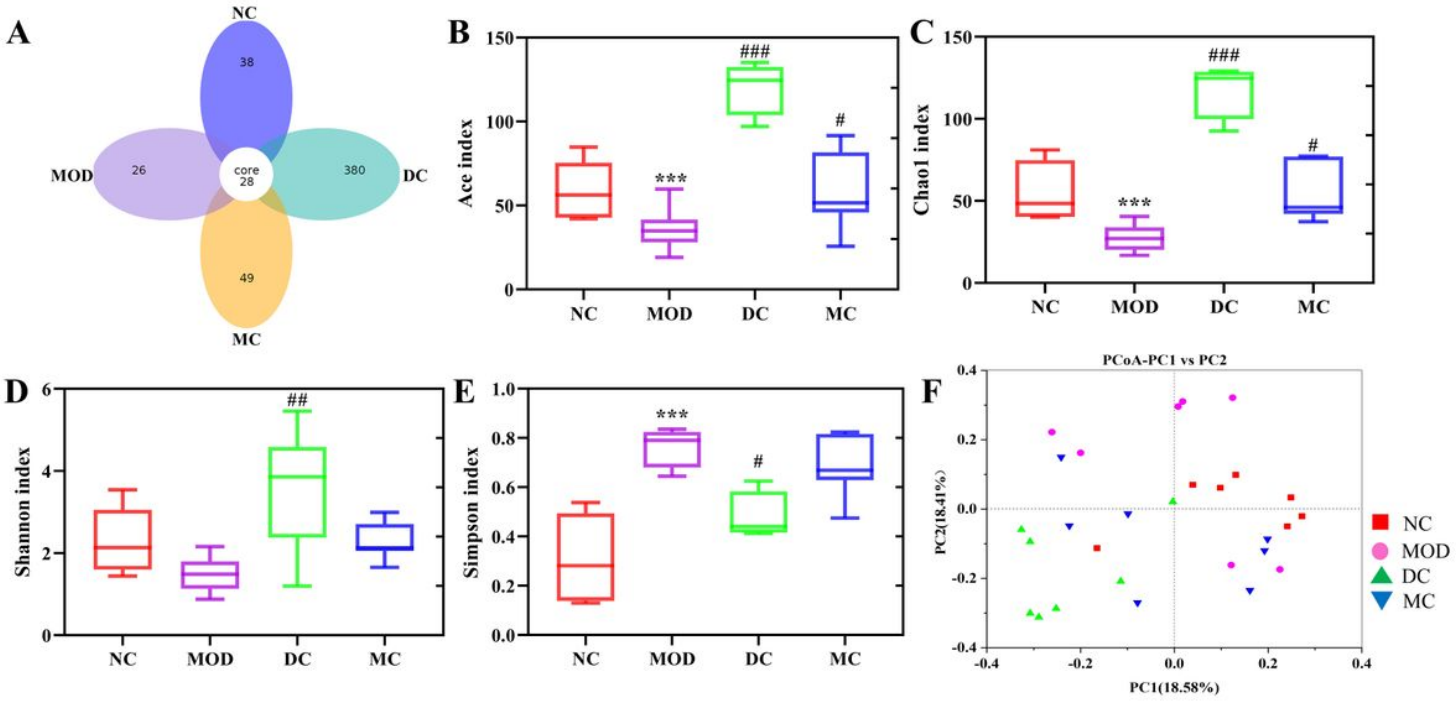

Figure 5

Effects of *Bacillus* sp. DU-106 on the diversity, structure, and composition of gut microbiota in T2D mice. (A) Venn diagram, (B) Ace index, (C) Chao1 index, (D) Shannon index, (E) Simpson index, and (F) principal coordinates analysis. Data are expressed as mean ± SD (n=7); * *p* ≤ 0.05, ** *p* ≤ 0.01, *** *p* ≤ 0.001, MOD group vs. NC group; # *p* ≤ 0.05, ## *p* ≤ 0.01, ### *p* ≤ 0.001, DC group or MC group vs. MOD group.

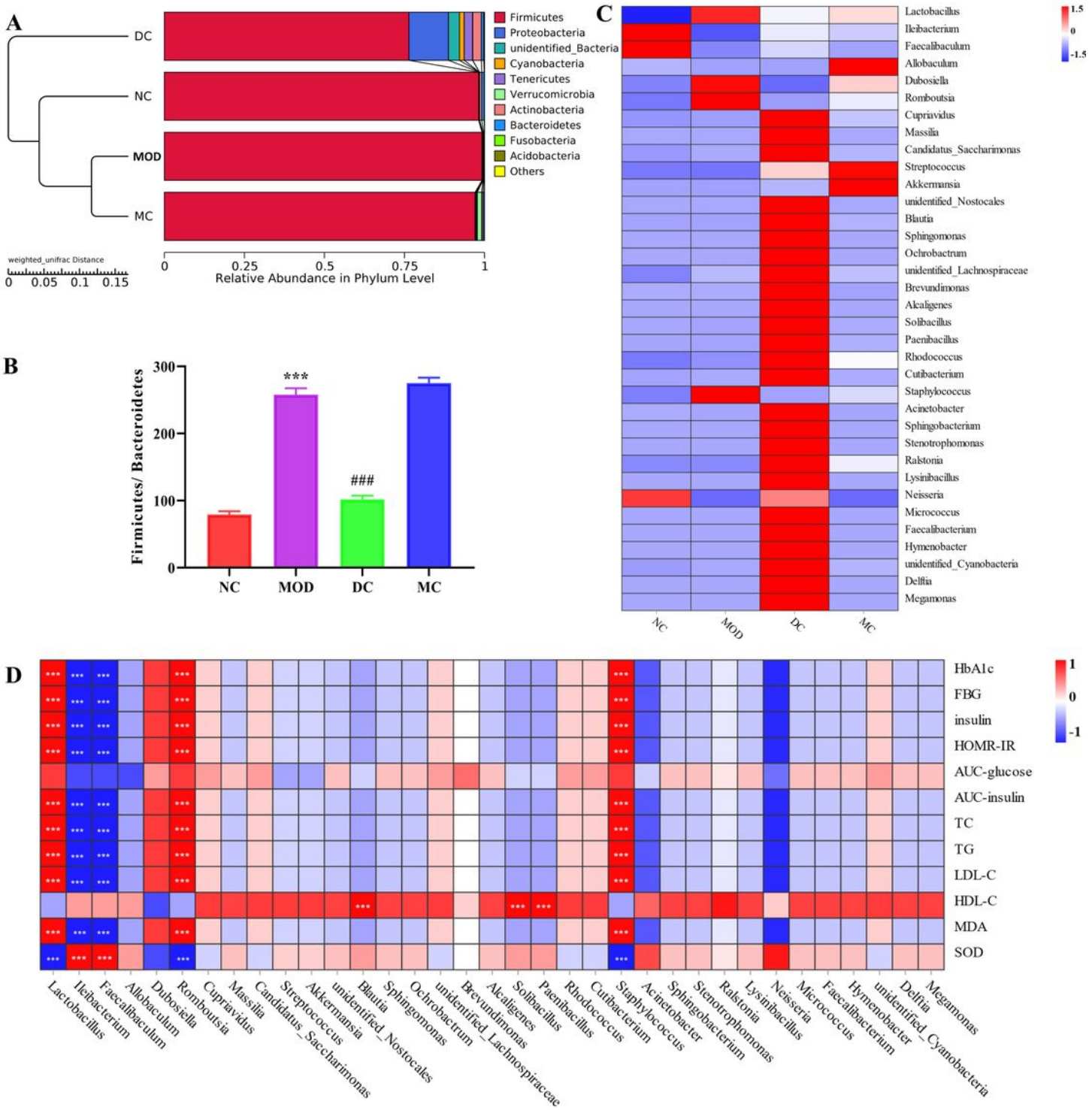

Figure 6

Effect of *Bacillus* sp. DU-106 on the taxonomic composition of gut microbiota in T2D mice. (A) Phylum level, (B) Firmicutes/Bacteroidetes ratio, (C) genus level, and (D) correlation between 35 genus level and biochemical parameters. Data are expressed as mean ± SD. Date are expressed as mean ± SD (n=7); * p < 0.05, ** p < 0.01, *** p < 0.001, MOD group vs. NC group; # p < 0.05, ## p < 0.01, ### p < 0.001, DC group or MC group vs. MOD group.